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Doklady Akademii Nauk SSSR, Novaya Seriya, Vol. LXIX, No. 5, 1949.

INFLUENCE OF CERTAIN SUBSTANCES UPON FREEZING PROCESSES  
AND THE REVIVESCENCE OF VITAL FUNCTIONS IN VERTEBRATA  
SUBJECTED TO FREEZING

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Much research has been done on the effect of various substances (sugars, alcohols, salts, etc.) on the resistance of plants to colds. Analogous studies have been made on infusoria, but as far as known, not on higher organisms in freezing temperatures. This article presents data on the effects of alcohol solutions on the resistance of a vertebrate animal against freezing temperatures and supplements those previous experiments described in Doklady Akademii Nauk SSSR, Vol. LIX, No. 2, 1943.

All experiments were conducted on male frogs (*Rana temporaria*) during the season (summer or spring) they were collected. The body temperature was thermoelectrically taken per rectum. Ethyl alcohol was introduced into the cerebrospinal canal and the lymphatic vessel.

### 1. Initial Freezing Temperature and Revivescence of Vital Functions after Freezing.

The frogs were fastened on their backs. The freezing process took place in a room where, in the course of the experiment, the temperature was lowered to  $-4$  to  $-6^{\circ}$ . The warming process took place in water at  $20^{\circ}$ . Two cubic centimeters of 40-percent alcohol were injected into some frogs  $\frac{1}{2}$  to  $1\frac{3}{4}$  hours prior to transfer into the freezing room. Those used for control were not injected. Previous experiments proved that the above-mentioned concentration and time intervals were best suited for such experiments.

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The results indicated that:

a. The alcohol solution inhibited the formation of ice within the body and lowered the initial freezing temperature from  $-1.0^{\circ}$  to  $-1.4^{\circ}$ . This temperature was as high as  $-1.2^{\circ}$  in only 17 percent of the frogs. A lower initial freezing temperature was obtained in these experiments with alcohol than in previous experiments on partly desiccated frogs.

b. The corresponding drop in body temperature due to supercooling reached  $-3$  to  $-5^{\circ}$ .

c. Complete and permanent revivescence of the main vital functions of the treated frogs was possible even after the body temperature registered lower than  $-1^{\circ}$ , but this was not obtained in the control frogs after the temperature dropped to  $-1.0$  to  $-1.5^{\circ}$ . Such revivescence was possible in ten out of 14 frogs which indicated from  $-1$  to  $-1.2^{\circ}$ ; in 6 out of 8 which indicated  $-1.2$  to  $-1.4^{\circ}$ ; in 4 out of 13 which indicated  $-1.4$  to  $-1.6^{\circ}$ ; but in no case at  $-2^{\circ}$ .

Consequently,  $-1.5^{\circ}$  can be considered close to the minimum body temperature which "alcoholized" frogs can resist (if the initial freezing temperature was  $-0.9^{\circ}$  or lower).

## 2. Dilatometric Experiments

Canals, similar in structure to those utilized by N. L. Sakharov in his experiments on insects, were filled with vaseline oil instead of gasoline on the same principle as that employed by Moran in his tests on muscles.

The frogs were immobilized by introducing a needle into the spinal cord. The air was drawn out of the alimentary tract and lungs, which were then filled with vaseline oil. Dilatometers were cooled in a cryohydrate solution of  $H_2SO_4$  down to  $-1.5^{\circ}$ . The level of the oil in the measuring tube (diameter 3 to 3.5 millimeters, error not exceeding 1/10 to 1/5 grams of water taken in calibrating the dilatometer) was recorded, first, in supercooling at  $-1.7^{\circ}$  and, again, at the same temperature, after freezing the frogs. Freezing was not caused by shaking, which proved ineffective, but by accelerating the cooling rate. The thermometer was placed in the esophagus.

Each experiment took from 1 to 2 days. The data obtained proved that 40-percent alcohol reduced the amount of frozen water obtained from the body of frogs subjected to  $-1.5^{\circ}$  temperature from 2.5 - 3 times as compared with the control specimens. For comparison, dilatometric calculations were made on frogs desiccated, by the method used in previous experiments, sufficiently to produce a 30- to 38-percent loss in weight, which corresponded to a 39- to 47-percent loss in water. In many cases the inhibition of ice formation was far more definite in "alcoholized" frogs than in desiccated frogs.

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